(FILE 'MEDLINE' ENTERED AT 16:07:15 ON 03 AUG 2004)

L1 10168 S HYPERPROLIFERATIVE? OR RESTENOSIS
L2 2341 S E2F
L3 11930 S RETINOBLASTOMA
L4 9 S L1 AND L2 AND L3
E ANTELMAN D?

FILE 'MEDLINE, CANCERLIT, SCISEARCH, CAPLUS, MEDICONF' ENTERED AT 16:19:34 ON 03 AUG 2004
L5 34 S L4
L6 16 DUP REM L5 (18 DUPLICATES REMOVED)
L7 16 FOCUS L6 1-

=> d an ti so au ab pi 17 1 2 3 12 14

L7 ANSWER 1 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
AN 2000:388523 CAPLUS
DN 133:54545
TI Sequences of **retinoblastoma**(Rb) and **E2F** fusion proteins and the therapeutic uses thereof for **hyperproliferative** disorders
SO U.S., 87 pp., Cont.-in-part of U.S. Ser. No. 751,517, abandoned.
CODEN: USXXAM
IN Antelman, Douglas; Gregory, Richard J.; Wills, Kenneth N.
AB The present invention relates to the construction of a chimeric gene encoding an **E2F** fragment containing DNA binding domain and nonfunctional cyclin A binding domain, and a Rb fragment including a functional growth suppression domain, wherein expression of the chimeric gene results in repressing transcription of **E2F** promoter, and causes cell cycle arrest in a variety of cell types. The invention also relates to operatively linking the fusion protein encoding DNA with a tissue-specific promoter such as a smooth muscle alpha actin promoter, to direct the tissue-specific cell growth inhibition. The invention further relates to the therapeutic uses of the fusion protein for the treatment of **hyperproliferative** disorders including cancer and **restenosis**.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI US 6074850	A	20000613	US 1997-801092	19970214
WO 9821228	A1	19980522	WO 1997-US21821	19971113
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9855899	A1	19980603	AU 1998-55899	19971113
AU 723660	B2	20000831		
EP 948520	A1	19991013	EP 1997-952238	19971113
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, LT, LV, FI, RO				
BR 9712767	A	19991026	BR 1997-12767	19971113
CN 1244870	A	20000216	CN 1997-181317	19971113
NZ 335738	A	20010223	NZ 1997-335738	19971113
JP 2001503638	T2	20010321	JP 1998-522958	19971113
CA 2271478	C	20030204	CA 1997-2271478	19971113
MX 9904499	A	20000531	MX 1999-4499	19990514
KR 2000053323	A	20000825	KR 1999-704327	19990515
US 6379927	B1	20020430	US 1999-315113	19990519

L7 ANSWER 2 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN
AN 1998:341586 CAPLUS
DN 129:36444
TI Transcription factor **E2F**-Rb protein fusions and tissue-specific

expression of **E2F**-Rb fusions in treatment of **hyperproliferative** diseases

SO PCT Int. Appl., 90 pp.
CODEN: PIXXD2

IN Antelman, Douglas; Gregory, Richard J.; Wills, Kenneth N.

AB Fusions of the transcription factor **E2F** and the **retinoblastoma** protein Rb are provided, along with methods of treatment of **hyperproliferative** diseases. 1-194- Or 1-286- **E2F** fused to 379-928-Rb protein containing A-606, A-612, A-788, A-807, and A-811 substitution mutations repressed transcription from an E2-CAT reporter construct 50-fold while the Rb protein mutant itself repressed transcription only 10-12-fold. An adenovirus vector expressing these fusion proteins from an actin promoter inhibited proliferation of smooth muscle cells.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9821228	A1	19980522	WO 1997-US21821	19971113
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US 6074850	A	20000613	US 1997-801092	19970214
AU 9855899	A1	19980603	AU 1998-55899	19971113
AU 723660	B2	20000831		
EP 948520	A1	19991013	EP 1997-952238	19971113
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, LT, LV, FI, RO				
BR 9712767	A	19991026	BR 1997-12767	19971113
NZ 335738	A	20010223	NZ 1997-335738	19971113
JP 2001503638	T2	20010321	JP 1998-522958	19971113
CA 2271478	C	20030204	CA 1997-2271478	19971113
MX 9904499	A	20000531	MX 1999-4499	19990514

L7 ANSWER 3 OF 16 CAPLUS COPYRIGHT 2004 ACS on STN

AN 1997:643967 CAPLUS

DN 127:314558

TI Adenoviral constructs encoding phosphorylation-competent full-length and truncated forms of the human **retinoblastoma** protein inhibit myocyte proliferation and neointima formation

SO Circulation (1997), 96(6), 1899-1905
CODEN: CIRCAZ; ISSN: 0009-7322

AU Smith, Roy C.; Wills, Ken N.; Antelman, Douglas; Perlman, Harris; Truong, Lonn N.; Krasinski, Kevin; Walsh, Kenneth

AB The **retinoblastoma** (Rb) protein is a key cell-cycle regulator that control entry into the S phase by modulating the activity of the **E2F** transcription factor. We analyzed the effects of full-length phosphorylation-competent and a mutant truncated form of human Rb for their effects on vascular smooth muscle cell (VSMC) proliferation and neointima formation. A number of mutant forms, both phosphorulation competent and incompetent, of human Rb protein were evaluated for their ability to inhibit **E2F** activity. The results of these assays indicated that a phosphorylation competent, amino-terminal-truncated Rb protein (Rb56) was a particularly potent inhibitor of **E2F** -mediated transcription relative to the full-length Rb construct (Rb110). Adenoviral constructs containing Rb56 or Rb110 expressed their resp. Rb forms in VSMCs, as determined by Western immunoblot anal., and were similar in their abilities to arrest the cell cycle, as determined by assays of 3H-thymidine incorporation and by flow cytometric analyses. When examined for their effect on neointima formation after balloon injury of the rat carotid artery, both full-length and truncated forms of Rb inhibited formation of this VSMC-derived lesion. These analyses revealed that the maintenance of high levels of phosphorylation-component human Rb, either full-length or truncated forms in VSMCs is in effective method of modulating the extent of intimal hyperplasia that occurs after balloon-induced vascular injury.

L7 ANSWER 12 OF 16 MEDLINE on STN
AN 1998080144 MEDLINE
TI Engineered mutants of pRB with improved growth suppression potential.
SO Oncogene, (1997 Dec 4) 15 (23) 2855-66.
Journal code: 8711562. ISSN: 0950-9232.
AU Antelman D; Perry S; Hollingsworth R; Gregory R J; Driscoll B; Fung Y K; Bookstein R
AB We have constructed a panel of substitution mutants which affect one or more of the putative cdk target sites of the RB protein. We have examined the activity of these mutants relative to wild-type RB by both a transcriptional repression assay and by measuring growth suppression in vitro. We find that some phosphorylation site mutants of pRB can repress E2 transcription more strongly than wild-type RB. These mutants are partially resistant to phosphorylation by cdks and can arrest tumor cells in G1 in vitro. Our results indicate a functional correlation between the ability to repress **E2F**-dependent transcription and the ability to suppress tumor cell growth in vitro. In addition, we describe two classes of RB mutants: N-terminal truncated p56RB and a novel mutant of RB containing multiple substitutions near its nuclear localization signal. Both classes of RB mutants have greater activity than the wild-type protein. Because RB is a key regulator of cell cycle progression, expression of a more potent, phosphorylation resistant RB may have utility in both RB(-/-) and RB(+/+) tumors as well as in **hyperproliferative** disorders.

L7 ANSWER 14 OF 16 MEDLINE on STN
AN 2001665750 MEDLINE
TI Gene therapy for cardiovascular disease: a case for cautious optimism.
SO Hypertension, (2001 Nov) 38 (5) 1210-6. Ref: 74
Journal code: 7906255. ISSN: 1524-4563.
AU Khurana R; Martin J F; Zachary I
AB There is currently intense interest in the development of gene therapy for cardiovascular disease. The stimulation of therapeutic angiogenesis for ischemic heart disease has been one of the areas of greatest promise. Encouraging results have been obtained with the angiogenic cytokines vascular endothelial growth factor (VEGF) and basic fibroblast growth factor in animal models, leading to clinical trials in ischemic heart disease. VEGF also has therapeutic potential in a second area of cardiovascular gene therapy, the enhancement of arterioprotective endothelial functions to prevent postangioplasty **restenosis** and bypass graft arteriopathy. The endothelial cell growth and survival functions of VEGF promote endothelial regeneration, whereas VEGF-induced endothelial production of NO and prostacyclin inhibits vascular smooth muscle cell proliferation. Inhibition of neointimal hyperplasia may also be achieved by gene transfer of endothelial NO synthase (eNOS), PGI synthase, or cell cycle regulators (**retinoblastoma**, cyclin or cyclin-dependent kinase inhibitors, p53, growth arrest homeobox gene, fas ligand) or antisense oligonucleotides to c-myb, c-myc, proliferating cell nuclear antigen, and transcription factors such as nuclear factor kappaB and **E2F**. An improved understanding of etiologically complex pathologies involving the interplay of genes and the environment, such as atherosclerosis and systemic hypertension, has led to the identification of new targets for gene therapy, with the potential to alleviate inherited genetic defects such as familial hypercholesterolemia. The use of vasodilator gene overexpression and antisense knockdown of vasoconstrictors to reduce blood pressure in animal models of systemic and pulmonary hypertension offers the prospect of gene therapy for human hypertensive disease. The renin-angiotensin system has been the target of choice for antihypertensive strategies because of its wide distribution and additional effects on fibrinolytic and oxidative stress pathways. Gene therapy in cardiovascular disease has an exciting future but remains at an early stage. Further developments in gene transfer vector technology and the identification of additional target genes will be required before its full therapeutic potential can be realized.

L Number	Hits	Search Text	DB	Time stamp
2	5220	retinoblastoma	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 16:31
3	585	retinoblastoma and E2F	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 16:30
4	171	(retinoblastoma and E2F) and (hyperproliferative OR restenosis)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 16:36
5	58	((retinoblastoma and E2F) and (hyperproliferative OR restenosis)) and (retinoblastoma WITH E2F)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 16:35
6	139910	hyperproliferative OR restenosis OR arterial OR artery OR blood ADJ vessel	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 16:37
7	2942	(hyperproliferative OR restenosis OR arterial OR artery OR blood ADJ vessel) and (E2F retinoblastoma rb56)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 16:38
8	37	((hyperproliferative OR restenosis OR arterial OR artery OR blood ADJ vessel) and (E2F retinoblastoma rb56)) and (E2F NEAR RB$2)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 16:40
9	42	((hyperproliferative OR restenosis OR arterial OR artery OR blood ADJ vessel) and (E2F retinoblastoma rb56)) and (E2F$2 NEAR RB$2)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 16:41
10	11	(E2F$2 NEAR RB$2).clm.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 16:41
11	11	(US-6379927-$ or US-6384299-$ or US-5759803-$ or US-5650287-$ or US-5532340-$ or US-5457049-$ or US-6074850-$ or US-6197756-$ or US-6596698-$ or US-6649158-$).did. or (WO-9821228-$).did.	USPAT; EPO	2004/08/03 17:08
-	269	E2F SAME (RB OR RB56)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/15 16:11
-	195	(E2F SAME (RB OR RB56)) and fusion	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/15 16:11
-	191	((E2F SAME (RB OR RB56)) and fusion) and (cancer or hyperproliferative or tumor)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/15 16:16
-	39	(((E2F SAME (RB OR RB56)) and fusion) and (cancer or hyperproliferative or tumor)) and (E2F NEAR RB)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/04/15 16:16
-	8	(US-5457049-$ or US-5532340-$ or US-5650287-$ or US-5759803-$ or US-5821070-$ or US-6379927-$ or US-6384299-$).did. or (WO-9821228-$).did.	USPAT; EPO	2003/04/15 16:20
-	4	Antelman NEAR Douglas	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/07/27 18:06
-	2	("6074850").PN.	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/07/27 18:06

-	4	Douglas NEAR antelman	USPAT; US-PGPUB; EPO; JPO; DERWENT	2004/08/03 15:26

1: Gene Ther. 2001 Dec;8(24):1847-54. Related Articles, Links

nature publishing group

Tissue-specific expression of an anti-proliferative hybrid transgene from the human smooth muscle alpha-actin promoter suppresses smooth muscle cell proliferation and neointima formation.

Wills KN, Mano T, Avanzini JB, Nguyen T, Antelman D, Gregory RJ, Smith RC, Walsh K.

Canji, Inc., San Diego, CA, USA.

The retinoblastoma protein (Rb), a key regulator of cell cycle progression, can bind the transcription factor E2F converting it from a positive transcriptional factor capable of driving cells into S phase into a negative complex which arrests cells in G1. We have created a potent transcriptional repressor of E2F-dependent transcription by fusing the C-terminal fragment of Rb (p56) to the DNA and DP1-binding domains of E2F. Because the expression of E2F/56 fusion protein from a constitutive promoter was incompatible with virus growth, adenovirus constructs were prepared where transgenes were expressed from a fragment of the smooth muscle alpha-actin (SMA) promoter. Immunoblot and beta-galactosidase staining demonstrated smooth muscle-specific expression of this transcriptional element in vitro. The SMA-p56 and SMA-E2F/p56 adenoviral constructs also induced G0/G1 cell cycle arrest specifically in smooth muscle cells. Following administration to rat tissues, the SMA-beta-galactosidase construct exhibited expression in balloon-injured carotid arteries, but not in liver, bladder or skeletal muscle. Local delivery of the SMA-E2F/p56 adenoviral construct to balloon-injured carotid arteries inhibited intimal hyperplasia. Our results demonstrate that local delivery of the SMA-E2F/p56 adenoviral construct can limit intimal hyperplasia in balloon-injured vessels, while avoiding toxicity that could occur from the dissemination and expression of the viral transgene.

PMID: 11821938 [PubMed - indexed for MEDLINE]

2: Oncogene. 1997 Dec 4;15(23):2855-66. Related Articles, Links

nature publishing group

Engineered mutants of pRB with improved growth suppression potential.

Antelman D, Perry S, Hollingsworth R, Gregory RJ, Driscoll B, Fung YK, Bookstein R.

Canji Inc., San Diego, California 92121, USA.

We have constructed a panel of substitution mutants which affect one or more of the putative cdk target sites of the RB protein. We have examined the activity of these mutants relative to wild-type RB by both a transcriptional repression assay and by measuring growth suppression in vitro. We find that some phosphorylation site mutants of pRB can repress E2 transcription more strongly than wild-type RB. These mutants are partially resistant to phosphorylation by cdks and can arrest tumor cells in G1 in vitro. Our results indicate a functional correlation between the ability to repress E2F-dependent transcription and the ability to suppress tumor cell growth in vitro. In addition, we describe two classes of RB mutants: N-terminal truncated p56RB and a novel mutant of RB containing multiple substitutions near its nuclear localization signal. Both classes of RB mutants have greater activity than the wild-type protein. Because RB is a key regulator of cell cycle progression, expression of a more potent, phosphorylation resistant RB may have utility in both RB(-/-) and RB(+/+) tumors as well as in hyperproliferative disorders.

PMID: 9419977 [PubMed - indexed for MEDLINE]

3: Circulation. 1997 Sep 16;96(6):1899-905. Related Articles, Links

Comment in:
- Circulation. 1997 Sep 16;96(6):1717-9.

Adenoviral constructs encoding phosphorylation-competent full-length and truncated forms of the human retinoblastoma protein inhibit myocyte proliferation and neointima formation.

Smith RC, Wills KN, Antelman D, Perlman H, Truong LN, Krasinski K, Walsh K.

Department of Medicine, Tufts University School of Medicine, St. Elizabeth's Medical Center, Boston, Mass 02135, USA.

BACKGROUND: The retinoblastoma (Rb) protein is a key cell-cycle regulator that controls entry into the S phase by modulating the activity of the E2F transcription factor. We analyzed the effects of full-length phosphorylation-competent and a mutant truncated form of human Rb for their effects on vascular smooth muscle cell (VSMC) proliferation and neointima formation. METHODS AND RESULTS: A number of mutant forms, both phosphorylation competent and incompetent, of human Rb protein were evaluated for their ability to inhibit E2F activity. The results of these assays indicated that a phosphorylation competent, amino-terminal-truncated Rb protein (Rb56) was a particularly potent inhibitor of E2F-mediated transcription relative to the full-length Rb construct (Rb110). Adenoviral constructs containing Rb56 or Rb110 expressed their respective Rb forms in VSMCs, as determined by Western immunoblot analysis, and were similar in their abilities to arrest the cell cycle, as determined by assays of 3H-thymidine incorporation and by

flow cytometric analyses. When examined for their effect on neointima formation after balloon injury of the rat carotid artery, both full-length and truncated forms of Rb inhibited formation of this VSMC-derived lesion. CONCLUSIONS: These analyses revealed that the maintenance of high levels of phosphorylation-competent human Rb, either full-length or truncated forms, in VSMCs is an effective method of modulating the extent of intimal hyperplasia that occurs after balloon-induced vascular injury.

PMID: 9323079 [PubMed - indexed for MEDLINE]
